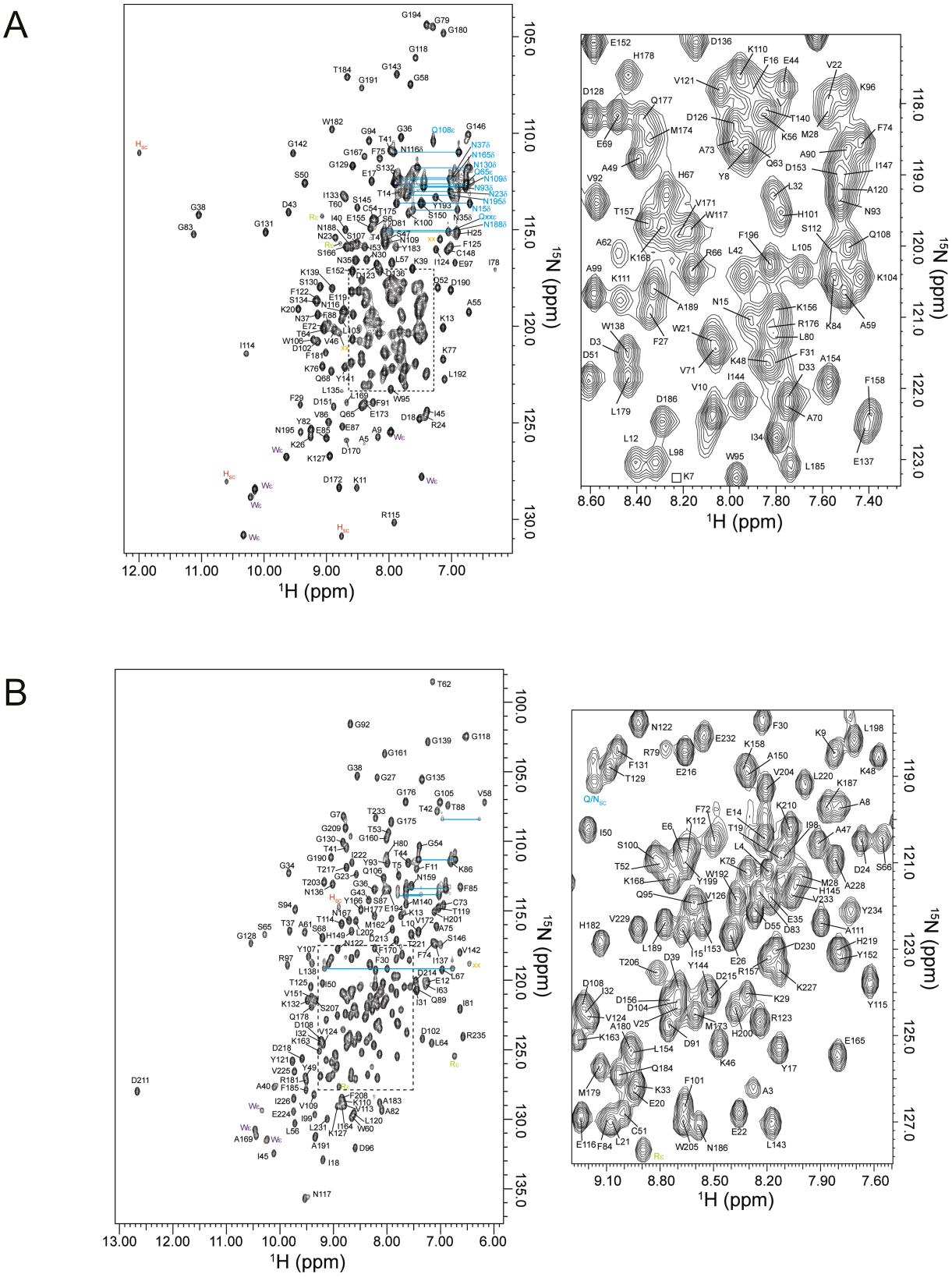
Supplementary information

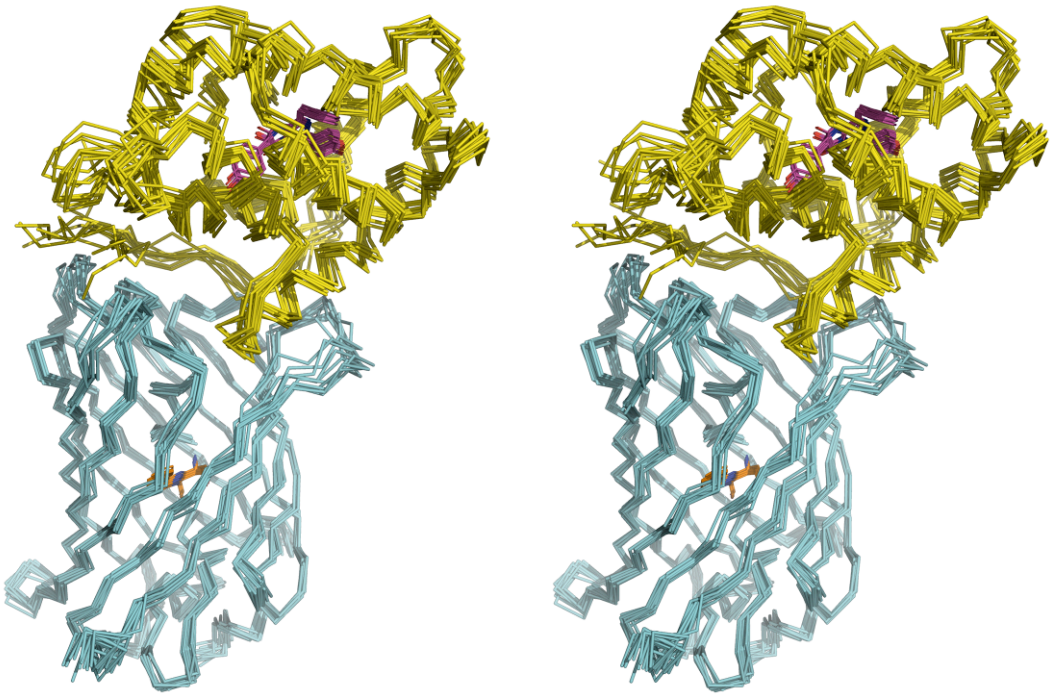
Supplemental Table S1: Parameters for 10 best HADDOCK score clytin-*cg*GFP structures

No.	HADDOCK score	E_{inter} (kcal/mol)	E_{vdw} (kcal/mol)	E_{elec} (kcal/mol)	E_{AIR} (kcal/mol)	# AIR Viol (>0.3 Å)	Buried Surface Area (Å^2)
1	-74.8967	-418.801	-74.6484	-470.344	126.191	3	2075.08
2	-67.5366	-421.660	-48.1242	-473.232	99.696	3	1776.07
3	-66.4241	-524.712	-44.4435	-531.243	50.974	2	1916.30
4	-59.1989	-370.435	-66.8908	-350.278	46.732	2	1877.61
5	-58.8372	-344.882	-56.8040	-431.780	143.702	3	1873.40
6	-58.2819	-380.386	-64.2805	-378.293	62.187	1	1960.47
7	-57.7164	-359.531	-56.3633	-409.118	105.950	2	2018.77
8	-56.9657	-300.890	-45.4271	-465.896	210.432	6	1834.81
9	-55.0314	-306.929	-61.3414	-377.973	132.386	3	1916.81
10	-54.9634	-399.903	-47.4098	-397.164	44.671	1	1885.00

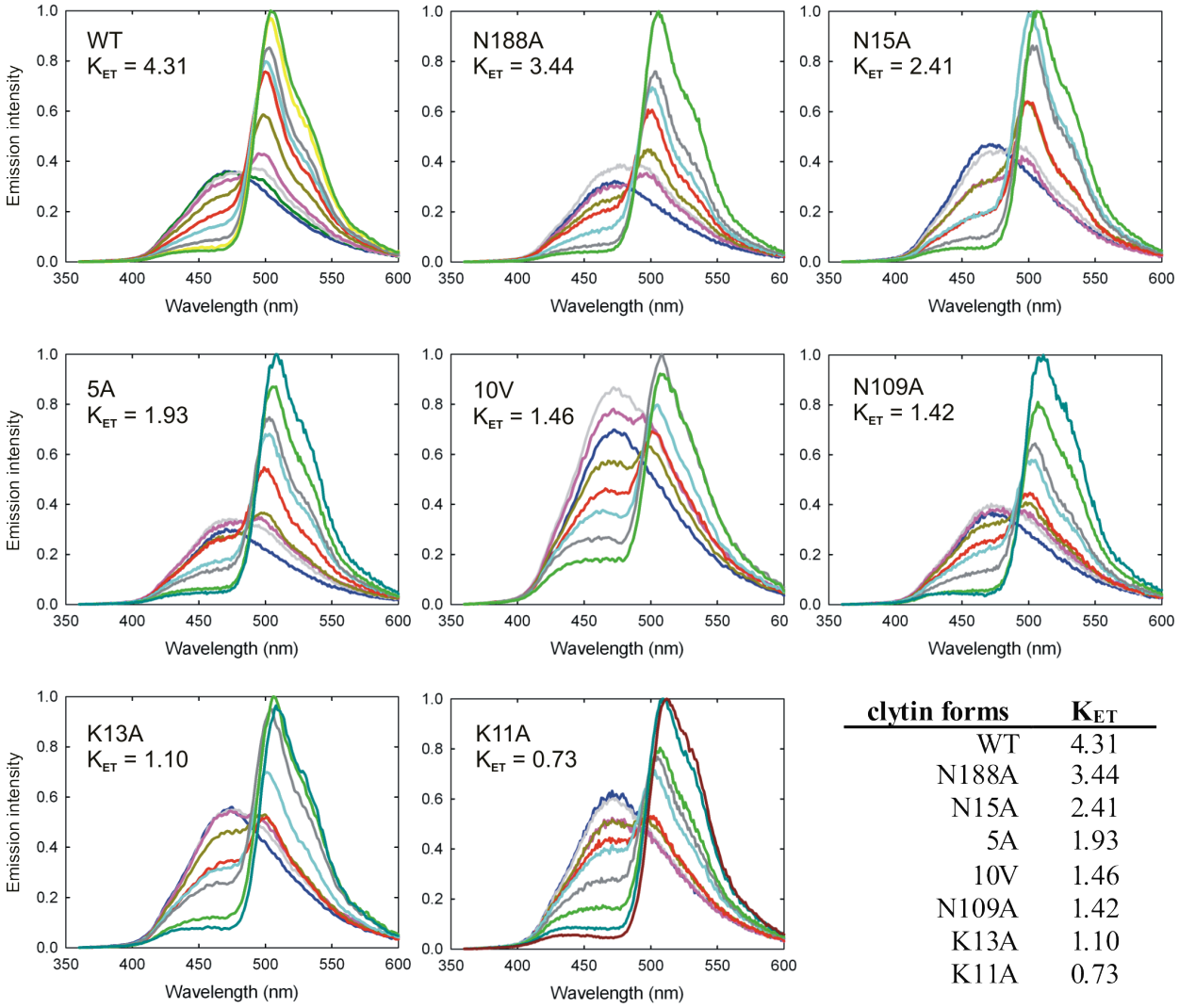
AIR Viol = number of AIR violations



Supplemental Fig. 1S: The assigned ^{1}H-^{15}N HSQC spectra of clytin (*A*) and *cg*GFP (*B*). Also shown are the peaks of side chain groups of Gln and Asn (*cyan*), Arg (*peridot*), His (*red*), and Trp (*purple*). Peaks without assignment are *orange*.

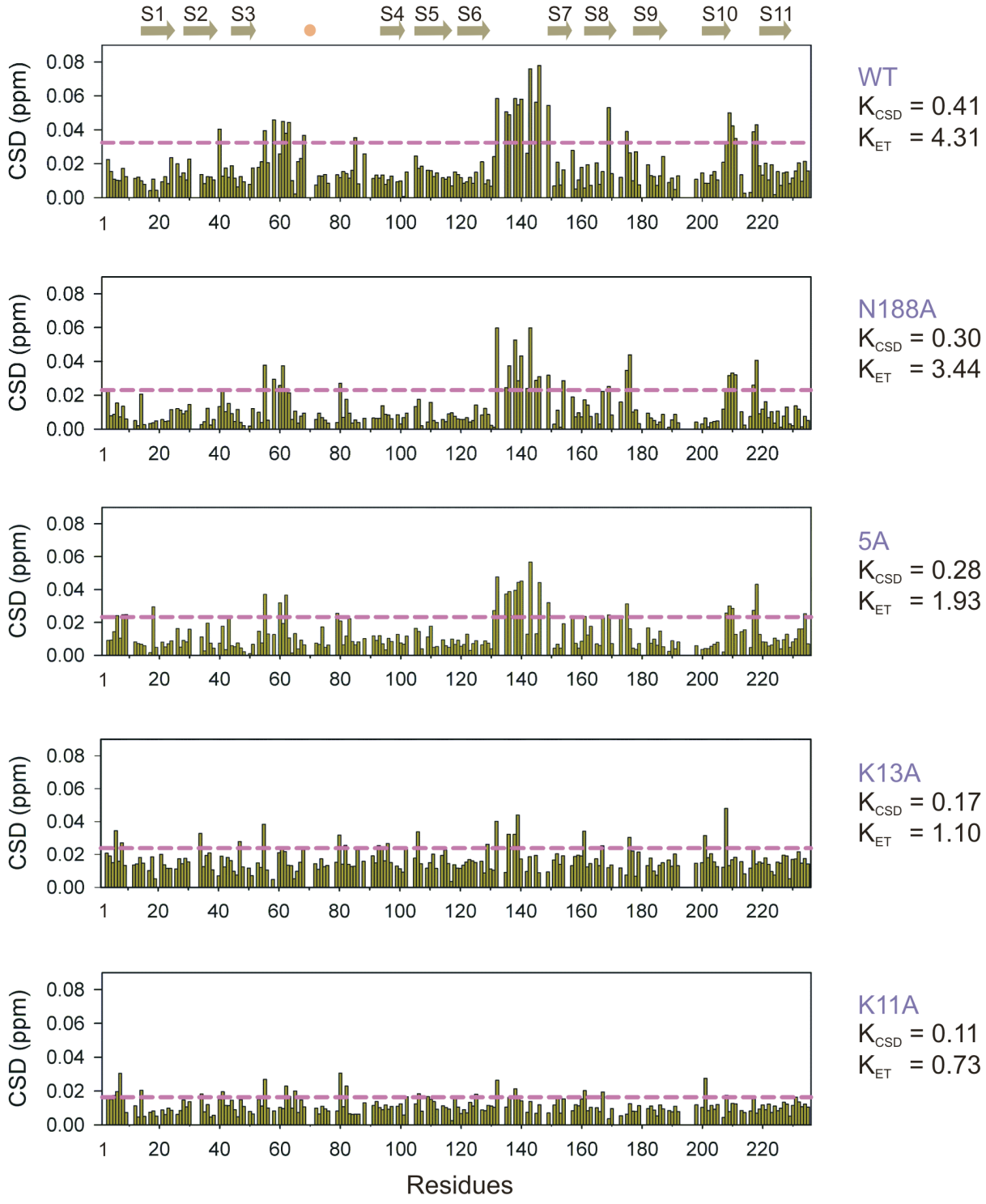


Supplemental Fig. 2S: Stereoview of the superposed ten lowest energy structures of the clytin-*cg*GFP complex generated by HADDOCK.

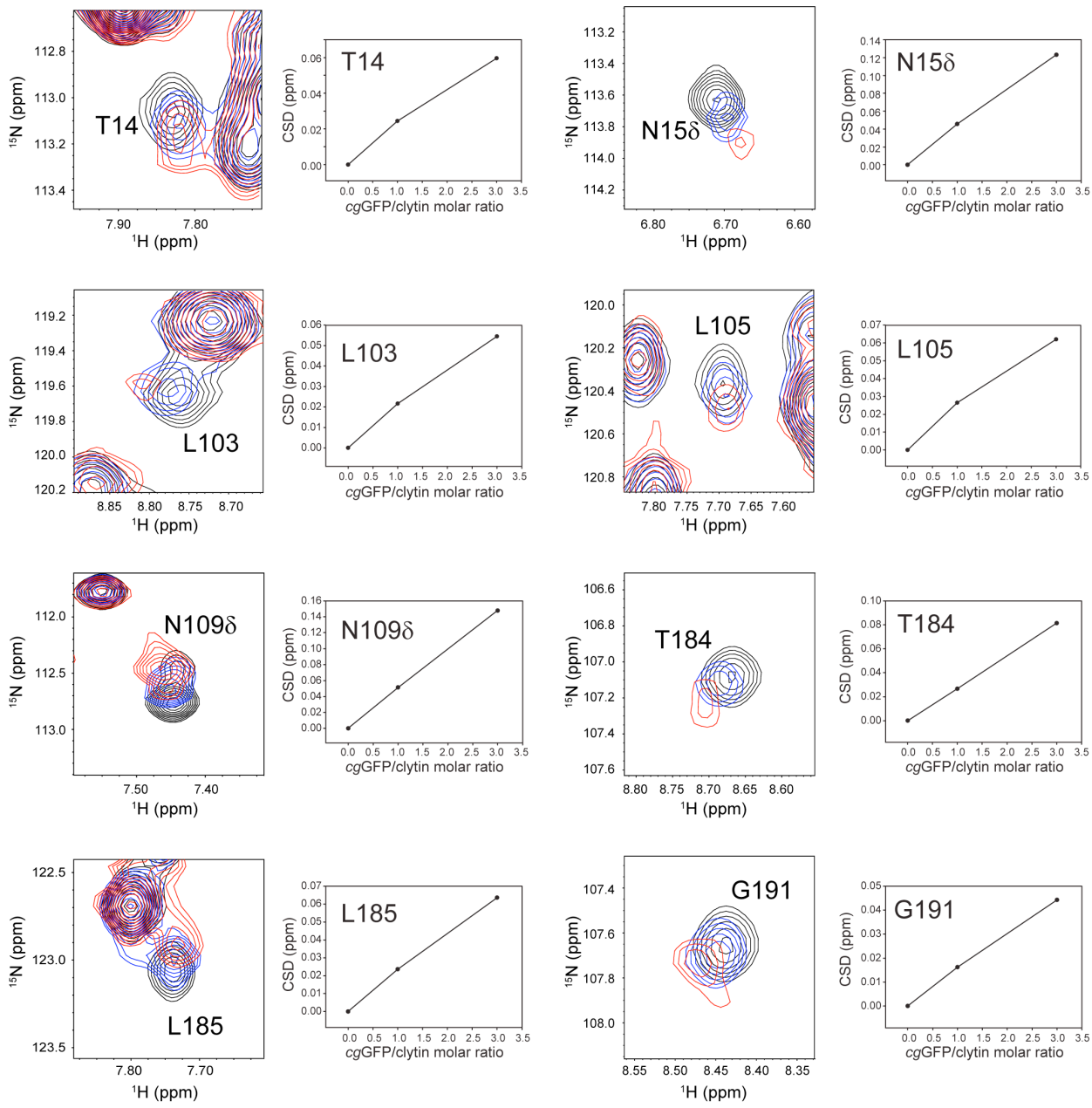


clytin forms	K_{ET}
WT	4.31
N188A	3.44
N15A	2.41
5A	1.93
10V	1.46
N109A	1.42
K13A	1.10
K11A	0.73

Supplemental Fig. 3S: Bioluminescence color-shift assay of clytin mutants showing the effect of mutation on the energy transfer measured as K_{ET}. The bioluminescence spectra were obtained upon titration with *cg*GFP (0–19.40 μM; 0 μM, 0.06 μM, 0.12 μM, 0.30 μM, 0.61 μM, 1.21 μM, 2.42 μM, 4.85 μM, 9.70 μM, 19.40 μM). For wild type (WT) clytin the color code is as follows: 0 μM, 0.03 μM, 0.06 μM, 0.12 μM, 0.30 μM, 0.61 μM, 0.90 μM, 1.80 μM, 3.00 μM, 4.24 μM of *cg*GFP. The concentration of clytin was in the 0.4–1.5 μM range. K_{ET} was determined as the slope of the I_{500}/I_{470} ratio versus *cg*GFP concentration, where I_{500} and I_{470} are bioluminescence intensities at 500 nm and 470 nm, respectively.



Supplemental Fig. 4S: Chemical shift perturbation plots of $^{15}N,^{2}H$-labelled *cg*GFP upon addition of 1 : 2 molar excess of wild type clytin or clytin mutants (N188A, 5A, K13A, K11A).
The corresponding K_{CSD} and K_{ET} values for each clytin form are shown. CSD = chemical shift difference. K_{CSD} was determined as a sum of CSD above the average CSD plus one standard deviation cut-off (*purple* dashed line) in ppm units.



Supplemental Fig. 5S: Superposition of ^{1}H-^{15}N HSQC spectra of ^{15}N-labelled clytin (0.2 mM) upon titration with *cg*GFP (0.2 mM or 0.6 mM, with the last value being close to the solubility limit) showing chemical shift perturbations of the single residues of clytin (T14, N15δ, L103, L105, N109δ, T184, L185 and G191). Clytin peaks are *black*; clytin peaks upon addition of *cg*GFP at 1:1 and 1:3 ratio are *blue* and *red*, respectively. The chemical shift differences (CSD) versus the molar ratio of *cg*GFP and clytin are plotted on the right of the corresponding zoomed in regions of ^{1}H-^{15}N HSQC spectra.

Supplemental Files:

clytin-cgGFP_haddockfit_1.pdb
clytin-cgGFP_haddockfit_2.pdb
clytin-cgGFP_haddockfit_3.pdb
clytin-cgGFP_haddockfit_4.pdb
clytin-cgGFP_haddockfit_5.pdb
clytin-cgGFP_haddockfit_6.pdb
clytin-cgGFP_haddockfit_7.pdb
clytin-cgGFP_haddockfit_8.pdb
clytin-cgGFP_haddockfit_9.pdb
clytin-cgGFP_haddockfit_10.pdb